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Procedia Food Science 3 (2015) 74 – 81

Procedia Food Science

International Symposium on Food and Agro-biodiversity

Isolation and Screening Bacterial Exopolysaccharide (EPS) from Potato Rhizosphere in Highland and The Potential as a Producer Indole Acetic Acid (IAA)

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Abstract

This study aimed to determine the ability of bacterial isolated from rhizosphere of potato plants in producing exopolysaccharide and growth stimulating substances such as Indole Acetic Acid (IAA).The soil samples were taken from three different land of slopes at elevation >1500 m above sea level at Malino,South Sulawesi. However, only 34 isolates formed a thick slime or mucoid when cultured onMacConcey medium.The ability of exopolysaccharide isolated bacterial in producing IAA was assayed in the presence of L-Tryptofan asa precursor.The result revealed that these 34 isolates were be able to produce IAA in range of 0.40 to 21.14 mg/L An isolate coded P2.67 was the most potential bacterium to produce IAA (21.14 ppm) followed by P2.56 (17.36 ppm), P3.42 (12.21 ppm), and P3.70 (9.21 ppm).

Peer-review under responsibility of the organizing committee of Indonesian Food Technologist Community

Keywords: exopolysaccharide, bacterial rhizosphere, potatoes, Indole acetic acid.

INRODUCTION

Intensive land use on horticultural crops in upland areas of high erosion is under focusing. Planting potatoes on sloping land is generally more usable to increase production, so that the land conservation issues are often ignored

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Peer-review under responsibility of the organizing committee of Indonesian Food Technologist Community
doi:10.1016/j.profoo.2015.01.007

[7]. Farmers seek many potato crops on slopes of 15% and 35% without attention to soil and water conservation which planting on ridges in the direction of the slope [11]. This condition leads to deterioration of land productivity, which will lower the potato production and farmers' income [7]. Therefore, efforts to maintain the productivity of the land by means of microbiological conservation, the use of microorganisms in the rhizosphere of potato plant roots which can improve soil structure by the effort of aggregating soil through microorganisms such are bacteria producing exopolysaccharide (EPS). Meanwhile, organic carbon particles improve soil aggregation when the material forming the organic core surrounded by clay, ash particles and aggregates [12]

Exopolysaccharide (EPS) is a complex mixture of macromolecular electrolyte contained on the outside of the bacterial cell is excreted as mucus that contributes to soil aggregation as an adhesive. Some exopolysaccharide-producing bacteria have been reported, among others, *Pseudomonas aeruginosa, Erwinia, Ralstonia,* and *Azotobacter vinelandii*. Exopolysaccharide protects bacteria from various environmental stresses [6], protects cells from antimicrobial compounds,and antibodies, or for sticking to other bacteria, animal and plant tissues [9,15]

Generally, plants are not able to produce IAA in sufficient quantities for growth and development. Some strains RPTT (Rizobacteria boosters grown plants) or popularly known PGPR (Plant growth promoting rhizobacteria) of precursors capable of synthesizing IAA (base material) contained in root exudates and organic ingredients. Various studies indicate that IAA produced by bacteria as PGPR *Azospirillum* and *Azotobacter paspali Brasiliense* can increase the number of lateral roots and root system size thereby increasing the water and nutrients uptake from the soil [1,8]. IAA synthesis in the ground had been available through specific precursors (base material) triptopan (L-tryptopan). Tryptopan is one source of N for the microbes contained in root exudates and organic matter can be converted by soil microbes into IAA [2]. This study was carried out to Isolation and Screening Bacterial Exopolysaccharide (EPS) from Potato Rhizosphere in Highland and The Potential as a Producer Indole Acetic Acid (IAA)

MATERIAL AND METHOD

Soil Sampling

Soil sampling have been taken for the purpose to isolate bacteria that producing exopolysaccharide (EPS) on rhizosphere potato plants at a depth of 0-20 cm with three gradients of the slope P1 (15%), P2 (25%) and P3 (35 %.). The type of soil samples is *Ultisol*. At each site soil samples taken in moderation, homogenized and put into a sterile plastic bag. Equipment have been cleaned and sterile with a wash and then rinsed or wiped with alcohol swabs.

Isolation and Purification of Bacteria producing exopolysaccharide (EPS)

Isolation of bacteria producing exopolysaccharide (BPE) was performed on several samples of soil were taken based on the slope is 15%, 25% and 35% in rhizosphere potato (*Solanum tuberosum* L) respectively. The depth of which Soil material had been taken was 0-20 cm. A total of one gram of soil material aseptically suspended in physiological saline solution (0.85%) and serial dilutions were made to 10-6, with Duplo and incubated in medium ATCC no. 14 (per liter of medium): 0.2 g KH_2PO_4; 0.8 g K_2HPO_4; 0.2 g $MgSO_4.7H_2O$; 0.1 g $CaSO_4.2H_2O$;

2.0 mg $FeCl_3$; $Na_2MoO_4.2H_2O$ (trace); 0.5 g extract yeast 20 g sucrose; and 15 g agar *bacto* with pH 7.2 and NB medium for seven days at a temperature of 28°C [10,11] Bacteria that produce EPS characterized by colonies of bacteria that form thick slime (*mucoid*) subsequently selected [14] and purified by streaking the four quadrants to obtain single colonies. Selection of bacterial exopolysaccharide-producing potential by setting the dry weight of bacterial exopolysaccharide produced according to the method proposed by [4].

Morphological identification and Gram Test with KOH

Identification of the bacterial groups is done through observation of colony morphology, the colony color, colony diameter and the diameter of the resulting slime and form colonies, gram test with KOH, to determine that the gram-negative bacteria produce exopolysaccharide Gram test using 3% KOH done by: Combine 1 loop pure cultures of bacteria with 1 drop of 3% KOH (w / v in H_2O), and then stirred repeatedly using a needle ose. Lift loopful many times from the suspension surface, observe whether the bacteria form a sticky suspension lifted like a thread with a needle ose. When the suspension turned been like a slimy, sticky and lifted like a thread with a needle ose, meaning Gram negative (-). Conversely, if the suspension remains dilute, not rose like the needle thread loop, meaning Gram positive (+). The selected bacterium is gram-negative bacteria. To prove and validate whether these bacteria are gram-negative bacteria producing exopolysaccharide subsequently grown on the Mac *Concey* medium (Oxoid CM 0007) is characterized by the presence of slime formed. [10].

Screening Bacteria Producing Exopolysaccharide

Selection and screening bacterial exopolysaccharide-producing potential by setting the dry weight exopolysaccharide produced by bacteria in liquid medium ATCC no. 14 (per liter of medium): 0.2 g KH_2PO_4; 0.8 g K_2HPO_4; 0.2 g $MgSO_4.7H_2O$; 0.1 g $CaSO_4.2H_2O$; 2.0 mg $FeCl_3$; $Na_2MoO_4.2H_2O$ (trace); 0.5 g Yeat Extrac, 20 g sucrose; with pH 7.2. Using sucrose as a carbon source method proposed by [4, 11]. Colonies of bacteria that form thick slime (mucoid) on solid medium no.14 ATCC were grown in 50 ml liquid medium ATCC no. 14 and incubated at a temperature of 28 ^{0}C for three days at the top of the machine shaker with 200 rpm rotation. At the end of incubation, cells were harvested with 1 mm EDTA by adding 500 µl, then shaken until homogeneous and then centrifuged at 9000 rpm for 10 min. The supernatant was separated from the bacterial cell deposition was taken, coupled with cold acetone solution with a ratio of 1: 3 [13]. Then have been performed again with the speed centrifugation 15000 rpm for 2 times 30 minutes. Deposition of biomass in the form of exopolysaccharide then washed with distilled water and dried at 60°C for 24 hours or until dry weights obtained were fixed.

Testing of IAA (Indol Acid Acetate)

To testing Bacterial that produced IAA production from exopolysaccharide-producing bacteria, NA media and the addition of L-tryptophan have been used. L-tryptophan is a precursor of IAA will provide a high production rate as reported [5]. Bacterial isolates were grown on NA medium for 72 hours at 28 ^{0} C in dark conditions. Bacteria that have grown up after 3 days of incubation were centrifuged at 3000 rpm for 30 minutes. Taken supernatant (2 ml) was mixed with two drops of orthophosphoric acid and 4 ml of *Salcowsci* reagent (50 ml, 35% of the sulfuric acid (H_2SO_4), 1 ml of 0.5 M solution of $FeCl_3$). To Indicated to IAA production by Pink discoloration showed. Optical

density was taken at 530 nm using a UV-Vis spectrophotometer. The concentration of IAA produced by bacteria was measured through a standard curve of pure IAA (Sigma-Aldrich) which obtained in the range of 10-100 mg / ml.

RESULTS AND DISCUSSION

The results recorded that there were 74 bacterial isolated obtained from the rhizosphere of potato plants originating from Malino, Subdistrict Moncong High, Gowa regency, South Sulawesi (Table 1). After further testing with 34 isolates of bacteria that have the potential to produce exopolysaccharide were grown on agar Mac Concey (medium selective for gram-negative bacteria) with the category of less level (+) to very good (++++) which form a thick slime (*mucoid*).

Figure 1 Bacterial Isolates producing exopolysaccharide

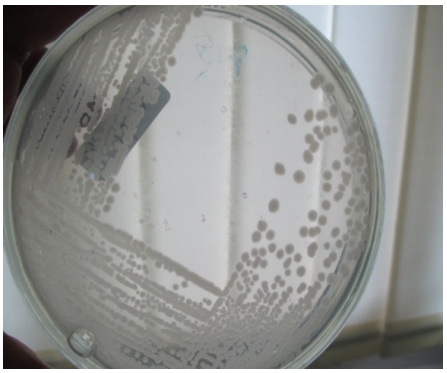
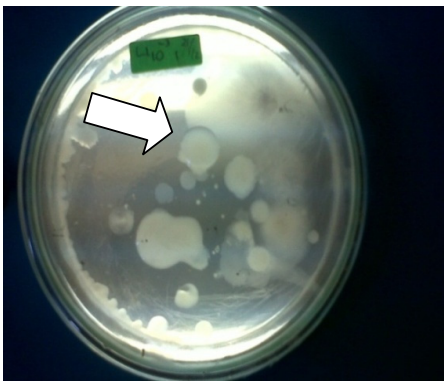


Figure 2. Formation of Slime (arrow) bacteria EPS on ATCC medium No. 14



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Isolation of exopolysaccharide-producing bacteria in the rhizosphere of plants potatoes made widely available in the soil matrix, moreover soil matrix is the development of roots, root exudates production plant metabolic results that contain lots of carbon compounds and the growth of macro and micro soil biota. As noted by [3] that the root exudates contain some organic compounds with low molecular weight such as simple sugars and polysaccharides (arabinose, lactose, glucose, maltose, mannose), oligosaccharides, amino acids (arginine, parangin, aspartate, Cysteine, cystine, glutamine), organic acids (acetic, ascorbic, benzoic acid and malic) and phenolic compounds. Some of these compounds have ability to enhance the growth and development of soil microorganisms.

Based on the results of measurements of dry weight exopolysaccharide (mg / ml) as shown in (Table 2), which investigated that the bacterial isolates coded P2 (37), P2 (57), P2 1 (60) and P3 (69) have the potential to be better when compared with other types of isolates. The dry weights of the four bacterial exopolysaccharide of potential were 1.75; 1.79; 1, 96 and 2.24 mg / ml of medium respectively.

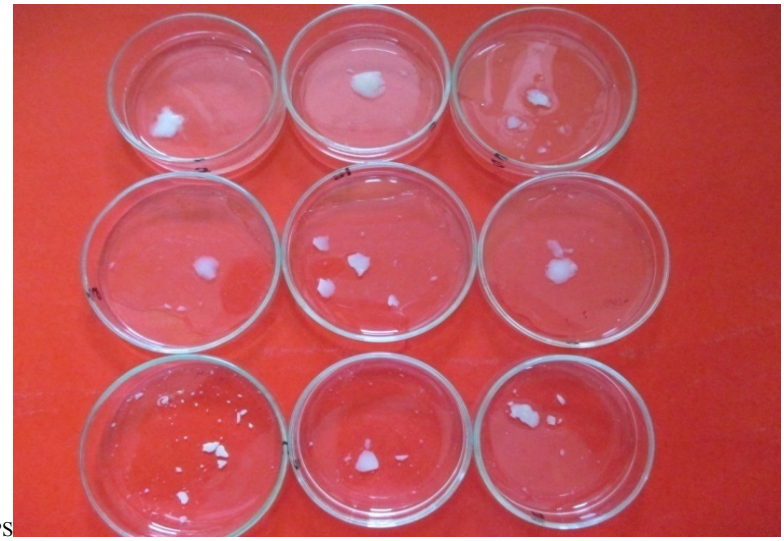
Table 1 Isolation of exopolysaccharide-producing bacteria from the potato rhizosphere

No.	Isolate Code	Colonies in agar color Mac Concey	Colony growth on medium Mac concey colonies 7 days	Diameter of colonies for 7 days (cm)	Gram Test
1	P1 (4)	Crème pink	+	0.1	Negative
2	P1 (6)	slightly pink	+++	0.3	Negative
3	P1 (7)	Crème pink	++	0.2	Negative
4	P2 (15)	Red	+	0.1	Negative
5	P2 (16)	Pink	+	0.1	Negative
6	P2 ((20)	Crème/Salem	++++	0.5	Negative
7	P2 (21)	slightly pink	++	0.2	Negative
8	P2 (27)	Transparent crème	++	0.2	Negative
9	P2 (32)	Transparent pink	+++	0.4	Negative
10	P2 (33)	Transparent crème	+++	0.3	Negative
11	P2 (34)	Transparent crème	+++	0,2	Negative
12	P2 (37)	Crème/salem	+++	0.3	Negative
13	P3 (38)	slightly pink	+	0.2	Negative
14	P3 (39)	Transparent white	++	0.2	Negative
15	P3 (41)	Transparent	+	0.2	Negative
16	P3 (42)	Transparent slightly pink	++	0.3	Negative
17	P3 (46)	slightly pink	+	0.2	Negative
18	P3 (48)	Transparent crème	++	0.2	Negative
19	P3 (49)	Transparent red	++	0,2	Negative
20	P3 (50)	slightly pink	++	0,2	Negative
21	P3 (51)	old pink	+++	0.4	Negative
22	P3 (53)	Transparent pink	+++	0.3	Negative
23	P2 (56)	Pink	++++	0.4	Negative
24	P2 (57)	slightly pink	++	0.2	Negative
25	P2 (58)	slightly pink	+	0.1	Negative
26	P2 (60)	Red	++	0.1	Negative
27	P2 (65)	Transparent pink	+++	0.2	Negative
28	P2 (6.6)	Old pink	+++	0.6	Negative
29	P2 (67)	Pink	++++	0.5	Negative
30	P3 (68)	Transparent pink	+++	0.3	Negative
31	P3 (69)	Transparent	++	0.1	Negative
32	P3 (70)	Red	+++	0.3	Negative
33	P3 (72)	Transparent	+++	0.2	Negative
34	P3 (73)	Transparent pink	+++	0.3	Negative

Table 2. Dry matter exopolysaccharide on exopolysaccharide production medium for 72 h of incubation

No	Isolate Code	Dry matter EPS (mg/ml)	No	Isolate Code	Dry matter EPS (mg/ml)
1	P2 (67)	0.87	18	P2 (16)	0.40
2	P1 (7)	0.45	19	P3 (51)	0.30
3	P3 (70)	1.67	20	P2 (66)	0.22
4	P3 (53)	0.30	21	P2 (34)	1.30
5	P3 (42)	0.10	22	P3 (50)	1.67
6	P3 (68)	0.45	23	P2 (37)	1.79
7	P2 ((20)	0,45	24	P3 (69)	2.24
8	P2 (65)	0.45	25	P1 (6)	1.04
9	P2 (56)	0.50	26	P2 (58)	0.54
10	P2 (32)	0.43	27	P3 (41)	1.52
11	P2 (27)	0.48	28	P3 (46)	1.17
12	P2 (21)	0.28	29	P3 (38)	1.70
13	P2 (33)	0.49	30	P3 (39)	0.8
14	P3 (49)	0.91	31	P2 (57)	1.75
15	P3 ((72)	0.32	32	P2 (60)	1.96
16	P3 (48)	0.33	33	P1 (4)	0.76
17	P3 (73)	0.57	34	P2 (15)	0.64

Figure 3 Production of exopolysaccharide by bacteria



EPS

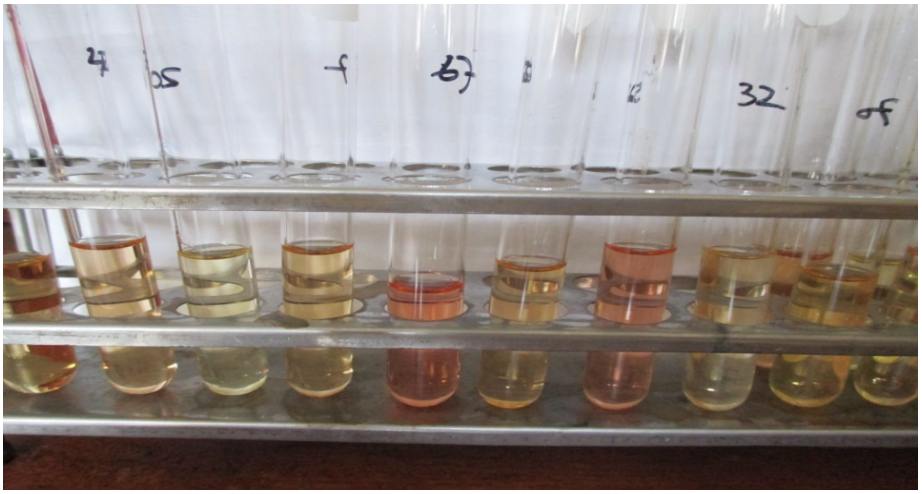
Four of potential bacterial exopolysaccharide producing each with code isolate P2 (37), P2 (57), P2 (60) and P3 (69) can produce dry weight exopolysaccharide from 1.75 to 2.24 mg / ml of medium. Dry weight exopolysaccharide weighing results indicated that the bacterial isolates code P3 (69) resulted in a higher dry weight of the bacterial isolates with other code. Bacteria excrete exopolysaccharide around the neighborhood growth. The amount and composition of the exopolysaccharide is highly variable depending on the genus and species of bacteria. Bacteria desperately need energy to produce exopolysaccharide. Therefore, the presence of a carbon source in the growth media can serve as a component in addition to the formation of cells may also serve as a source of energy that is required for exopolysaccharide synthesis and excretion [11]

Table 3: Production of IAA produced by bacteria producing exopolysaccharide

No	Isolat Code	Production of IAA (rpm)	No	Isolate Code	Production of IAA (rpm)
1	P1 (4)	1.29	18	P3 (48)	0.40
2	P1 (6)	2.40	19	P3 (49)	0.44
3	P1 (7)	9.21	20	P3 (50)	1.29
4	P2 (15)	0.90	21	P3 (51)	1.79
5	P2 (16)	0.64	22	P3 (53)	7,86
6	P2 ((20)	4.36	23	P2 (56)	17.36
7	P2 (21)	1.13	24	P2 (57)	1.22
8	P2 (27)	0.83	25	P2 (58)	3.00
9	P2 (32)	5.07	26	P2 (60)	2.87
10	P2 (33)	0.76	27	P2 (65)	7.79
11	P2 (34)	0.70	28	P2 (6.6)	1.71
12	P2 (37)	1.52	29	P2 (67)	21.14
13	P3 (38)	1.35	30	P3 (68)	8.79
14	P3 (39)	2.05	31	P3 (69)	1.78
15	P3 (41)	1.52	32	P3 (70)	9.21
16	P3 (42)	1.21	33	P3 (72)	1.27
17	P3 (46)	0.73	34	P3 (73)	2.06

Bacterial isolates with code P2 (67) is the most of potential isolates produce IAA production (21.14 ppm) followed P2 (56): (17.36 ppm), P3 (42): (12.21 ppm), and P3 (70), namely (9.21 ppm). Indole acetic acid is the active form of the hormone auxin found in plants and contributed to improve the quality and yields. Functions for the plant hormone IAA including increased cell growth, stimulate the formation of new roots, stimulate flowering, increasing the activity of the enzyme [2].

Figure 3 Production of IAA produced by bacterial exopolysaccharide



Four isolates bacteria have the value of potential to producing exopolysaccharide, isolates code P3 (69) exopolysaccharide producing high amounts of 2.24 mg / ml compared with other isolates. Similarly yielding bacterial IAA production of potential there are four isolates, isolates code P2 (67) produces the highest-value IAA for 21.14 ppm. Bacterial isolates that produce exopolysaccharide and IAA production seen from the origin of the samples derived from the slope of bacteria both 25% and 35%

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Presented at ISFA (September 16-17, 2014-Semarang, Indonesia) as Paper #26 "Managing Biosafety and Biodivesity of Food from Local to Global Industries"